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Patentanmeldung Nr. Patent application No. Demande de brevet n°

99117506.8

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System for thermocycling of fluids in cartridges

This invention is directed to a system for thermocycling fluids in cartridges to achieve an amplification and detection of nucleic acid sequences. A specific aspect of the present invention is that amplification and monitoring of the amplification process can be made simultaneously without changing the position of the cartridge. The monitoring of the amplification process may be used to quantitate the starting concentration of a target nucleic acid.

An apparatus for performing nucleic acid amplifications in reaction cartridges is known from US 5,567,617. This U.S. patent is concerned with an invention for nucleic acid amplification in a flexible cuvette. For amplification this cuvette is placed within a heater having a thin heating element with a central window providing an optical passage. Such an embodiment has the disadvantage that heating and detection compete for free space in the lateral direction of the reaction cuvette. A further problem of the system used in US 5,567,617 is the flexible nature of the cuvette with fluid channels therein. Measures have to be taken to ensure that fluid communication within the cuvette is not obstructed when the cuvette is placed within the heater.

The present invention is directed to a system for thermocycling similar to the system described in US 5,567,617 but having the advantage that heating efficiency is not limited by free space needed for detection. A further advantage compared to US 5,567,617 is that a constriction of fluid passways within the cartridge is uncritical.

The system of the present invention comprises a thermocycling unit, a light source, a light detector, a fluid providing unit, and a cartridge in which thermocycling as well as detection can be conducted while the cartridge remains unchanged in position.

It should be noted that the interior of the cartridge may have fluid channels, as well as protrusions and recesses. Furthermore the used language "closed cartridge" does not exclude fluid channels intersecting the walls of the cartridge which are used for bringing fluids into or out of the cartridge.

The thermocycling unit of the present invention comprises a heating section for establishing a thermal contact with the heat conducting walls of the cartridge. The heating section preferably comprises at least one plate which is brought into mechanical contact with the heat conducting wall of the cartridge and the plate itself being heated and cooled as necessary for the thermocycling process. This heating and cooling can be made by e.g. peltier elements, bringing the plate into contact with hot and cold fluids or by heating with a resistor heater and cooling by blowing air onto the plate. Procedures and apparatuses for performing thermocycling by thermal contact with plates are well known in the art.

It should be understood, however, that other devices for heating and cooling the cartridges can be used without departing from the scope of the invention. It is only necessary that whatever device is used for heating and cooling the cartridges, be capable of reaching and sustaining the temperatures involved and achieve the desired temperature versus time profile. Thus, for purposes of nucleic acid amplification, such a device should be capable of cycling the temperature of the amplification reaction mixture between a denaturing temperature T_1 (which can be in the range of about 80-105°C and preferably 90-100°C) and an annealing/extension temperature T_2 (which can be in the range of about 30-90°C and preferably 50-70°C) where $T_1 > T_2$ as is known to those skilled in the art.

To achieve a sufficient thermal contact between the cartridge and the heating section means can be provided which press one or two plates against the cartridge. Such means are e. g. described in US 5,567,617. In the context of the present invention it is however preferred to employ a heating unit having a receiving section of a wedge shaped recess. The receiving section may be formed by two walls which are inclined one to the other. A wedge shaped cartridge can be simply placed into such a wedge shaped receiving section and sufficient thermal contact between the walls of the receiving section and the heat

the exponential accumulation of the specific target fragment, at a rate of approximately 2^n per cycle, where n is the number of cycles. A complete review of this technology can be found in PCR Technology- Principles and Applications, Ed. Erlich H.A., Stockton Press, Now York 1989. Taq DNA polymerase is preferred when PCR is used in conjunction with the present invention although this is not an essential aspect of the invention.

The ligase chain reaction is described in International Patent Application WO 89/09835. The process involves the use of ligase to join oligonucleotide segments that anneal to the target nucleic acid. Ligase chain reaction (LCR) results in amplification of an original target molecule and can provide millions of copies of product DNA. Consequently, the LCR results in a net increase in double-stranded DNA. The present detection methods are applicable to LCR, as well as PCR. LCR typically requires some means for detecting the product DNA such as an oligonucleotide probe. When used in conjunction with the disclosed methods for detecting amplification products, such means are unnecessary, and the LCR result is immediately detectable.

Another amplification scheme, Q-beta replicase, exploits the use of the replicase from the RNA bacteriophage Qß. In this amplification scheme, a modified recombinant bacteriophage genome with a sequence specific for the targeted sequence is initially ligated to the nucleic acid to be tested. Following enrichment of the duplexes formed between the bacteriophage probe and the nucleic acid in a sample, Qß replicase is added, which, upon recognizing the retained recombinant genome, begins making a large number of copies. The Qß system does not require primer sequences and there is no heat denaturation step as with the PCR and LCR amplification systems. The reaction occurs at one temperature, typically 37°C. The preferred template is a substrate for the Qß replicase, midvariant-1 RNA. A very large increase in the templates is achieved through the use of this system. A review of this amplification system can be found in International Patent Application WO 87/06270 and in Lizardi et al., 1988, Bio/Technology 6:1197-1202.

The 3SR system is a variation of an in vitro transcription-based amplification system. A transcription-based amplification system (TAS) involves the use of primers that encode a promoter sequence as well as a complementary sequence to the target strand to generate

present invention. Suitable donor fluorophores and quenchers are chosen such that the emission spectrum of the donor fluorophore overlaps with the absorption spectrum of the quencher. Ideally, the fluorophores should have a high Stokes shift (a large difference between the wavelength for maximum absorption and the wavelength for maximum emission) to minimize interference by scattered excitation light.

Suitable labels which are well known in the art include, but are not limited to, fluoroscein and derivatives such as FAM, HEX, TET, and JOE; rhodamine and derivatives such as Texas Red, ROX, and TAMRA; Lucifer Yellow, and coumarin derivatives such as 7-Me2N-coumarin-4-acetate, 7OH-4-CH3-coumarin-3-acetate, and 7-NH2-4-CH3coumarin-3-acetate (AMCA). FAM, HEX, TET, JOE, ROX , and TAMRA are marketed by Perkin Elmer, Applied Biosystems Division (Foster City, CA). TEX-as Red and many other suitable compounds are marketed by Molecular Probes (Eugene, OR). Examples of chemiluminescent and bioluminescent compounds that may be suitable for use as the energy donor include luminol (aminophthal-hydrazide) and derivatives, and Luciferases.

The optics of a system in accordance with the present invention comprises a light source and a light detector . With the system absorption or scattering measurements with the fluid within the cartridge can be performed. It is however preferred to use this system for fluorescent measurements where light is transmitted into the interior of the cartridge to initiate fluorescence emission which is detected by the light detector. The light source may comprise semi-conductor light sources as well as halogen lamps or other embodiments. Within the spirit of the present invention the light is transmitted into the cartridge through a second light transparent wall of the cartridge which is substantially perpendicular to the wall for heat transfer. Due to the flat shape of the cartridge the second light transparent wall has a width of only 0,5 to 5 mm in one dimension. It is therefor preferred to employ beam shaping optics coorporating with the light source to introduce light through this restricted window. Such beam shaping optics may include apertures, lenses and fibre optics. For fluorescent measurements it is necessary to stimulate fluorescent dyes with light of a wavelength within the absorption spectrum of the dye. It is normally desired to suppress background radiation caused by light emission from other sources than the fluorescent dye. In many embodiments it is furthermore desirable to perform fluorescent

receiving unit is necessary if the cartridge has to be emptied or if different fluids have to be processed within the same cartridge. In preferred embodiments the cartridge is however discarded together with the fluid therein after single use. Such embodiments do not need a fluid receiving unit but only an outlet to drain gas when the cartridge is filled with fluid.

The present invention is further described in more detail with the regard to the following figures:

Figure 1: Cartridge in top view partially filled with fluid

Figure 2: Cartridge in top view with light sources and detector

Figure 3: Light source module

Figure 4: Raytrace diagram with cartridge having an oblique window

Figure 5: Fluorescence signal during thermocycling

Figure 6: System for thermocycling in a cartridge within a metal block thermocycler, integrating light sources and light detector.

Figure 7: System for thermocycling with wedge shaped cartridge

Figure 1 shows a cartridge (1) for thermocycling of fluids in top view. The cartridge has a body (3) with a cavity (2) therein. The cartridge body has been molten from a solid polypropylene block. In the region of the cavity material was removed until a cell bottom of 200µm thickness had been achieved. (In serial production the cartridge can be produced more efficiently by injection molding.) Into the polypropylene block has also been molten an inlet (10) to receive fluid and an outlet (11) to vent air from the cavitiy during filling. The body of the cartridge shown in figure 1 has been closed by welding a sealing foil of 200µm thickness onto the cartrigde body. Figure 1 shows a preferred shape of the cavity (2) with a protrusion (5) in form of a nose reaching into the cavity. The protrusion (5)

the light sources the amount of each fluorescent dye can be monitored by detection of fluorescent radiation with the detector.

Figure 3 shows a light source module which can be advantageously used within the present invention. The light source module comprises a semiconductor light source (31) a wavelength selection filter (32) and an optical output power monitor (33). The output power monitor comprises a beam splitter (33a) to extract a portion of the light from the light path and a detector (33b) to detect the extracted light. The amount of light detected is used to steer the current applied to the semiconductor light source so as to generate a constant and specific output. The light source module of figure 3 further comprises a beam shaper (34) in form of a lens. Advantageously the light emitting surface of the semiconductor light source is imaged by the lens into the centre of the cartridge.

Figure 4 shows a raytrace of a system employing a cartridge with an oblique window. As can be seen the light generated by light source (30) is imaged by a ballshape lens (35) onto an oblique window (4) of the cartridge. Due to this rangement the light is refracted in direction of the detector (20).

Figure 5 depicts a fluorescence over time diagram which was measured with the system according to the present invention. The abscissa shows the number of the measurement and the ordinate shows fluorescence intensity in arbitrary units. Measurements were taken after each denaturation, elongation cycle of the polymerase chain reaction. A cartridge (1) as shown in figures 1 and 6 has been introduced and fixed in a holder as shown in figure 6. The holder with the cartridge had been integrated into a thermocycler unit. Illumination optics with simple beam shaping optics and detection optics have been used for quantitative fluorescence measurement. PCR runs (HCV-control with Mastermix, 5×10^3 initial copies) with the following protocol have then been performed: 120 cycles, denaturation tempearature $T_{denat} = 94°C$, annealing temperature $T_{anneal} = 60°C$, tempearture ramp time t_{ramp} approx. 20 s, tempearture plateau $t_{plateau}$ approx. 26 s, total process time t_{tot} approx. 3 h. The experimentally measured fluorescence signal vs. number of measurement is plotted in Fig. 5 (solid line). For comparison, the fluorescence signal of an amplification within a vessel has been measured with the same PCR protocol. The result is also shown in

Claims

1 System for thermocycling of fluids in cartridges comprising

a) a thermocycling unit in thermal contact with a first substantially planar and heat conducting wall of a cartridge (1),

b) a light source (30) for transmitting light into the interior of said cartridge through a second light transparent wall of said cartridge which is arranged substantially perpendicular to said first wall,

c) a light detector (20) for detecting light emerging from the interior of the cartridge through said second wall,

d) a fluid providing unit coupled to an inlet of the cartridge for providing the cartridge with fluid.

2. System according to claim 1, wherein the cartridge has a body comprising the second wall and having at least one opening which is sealed by a foil providing said first heat conducting wall.

3. System according to claim 2, wherein the body is a frame which is sealed by two foils providing heat conducting walls.

4. System according to claim 1 for conducting fluorescent measurements wherein the light detector detects fluorescent light emerging from the cartridge.

5. System according to claim 1, wherein the thermocycling unit comprises at least one plate (40) in thermal contact with the first wall of the cartridge.

6. System according to claim 1, wherein the cartridge is wedge shaped and the thermocycling unit comprising a wedge shaped receiving section for receiving said wedge shaped cartridge.

14. Cartridge according to claim 11 or 12, having two opposing walls from which at least one is a heat conducting wall and which form an angle of 3 to 8° with respect to each other.

15. Cartridge according to claim 11, wherein the second wall comprises a first section for transmitting light into the cartridge and a second section for transmitting light emerging from the cartridge.

16. Cartridge according to claim 11, which is made from a body having at least one opening which is closed by a heat conducting foil.

17. Cartridge according to claim 11, which is made from a frame which is closed by two opposing foils.

18. Cartridge according to claim 11 having a thickness of 0,5 to 5 mm.

Fig 1

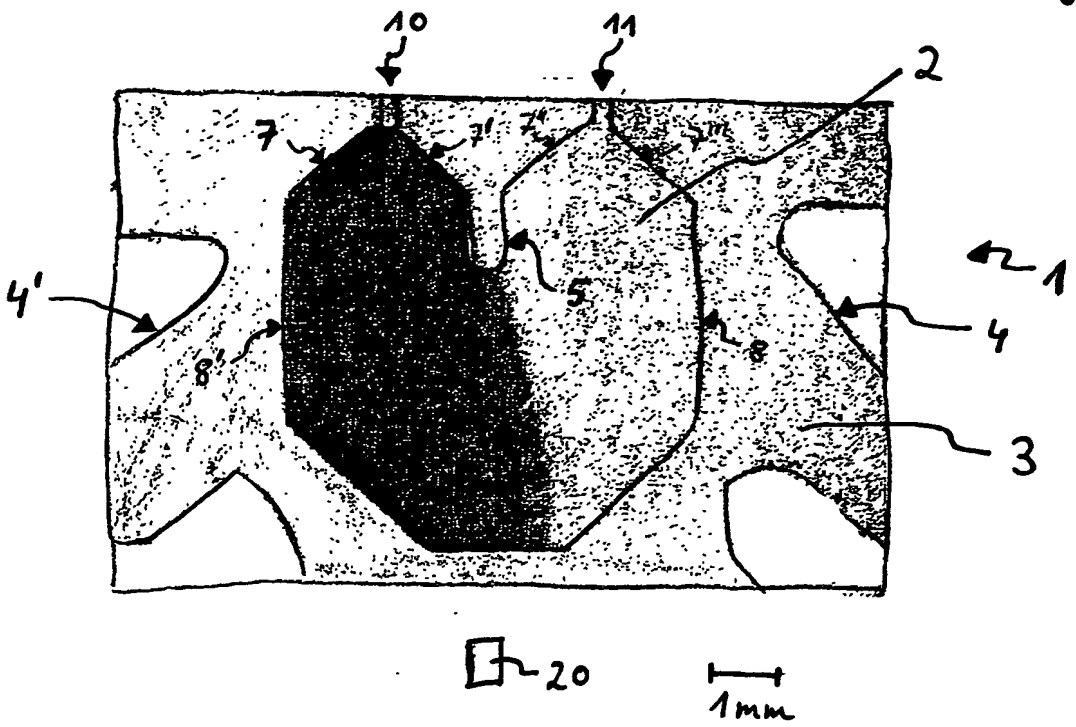


Fig 2

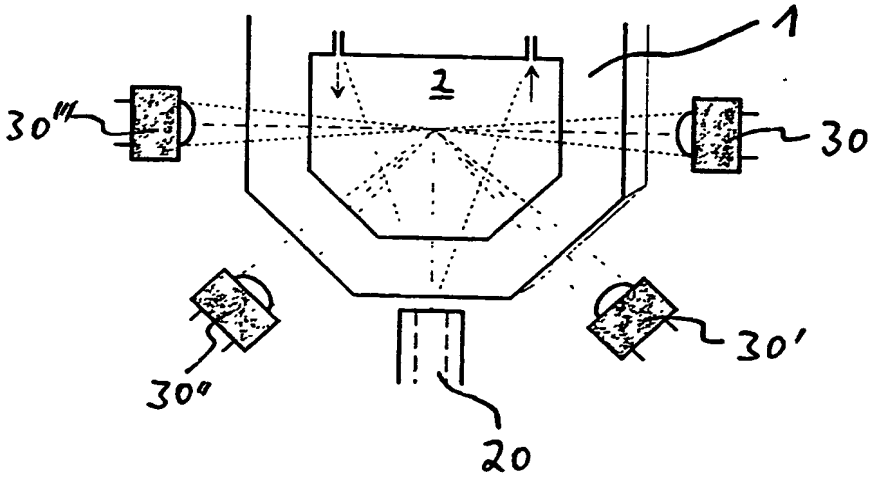
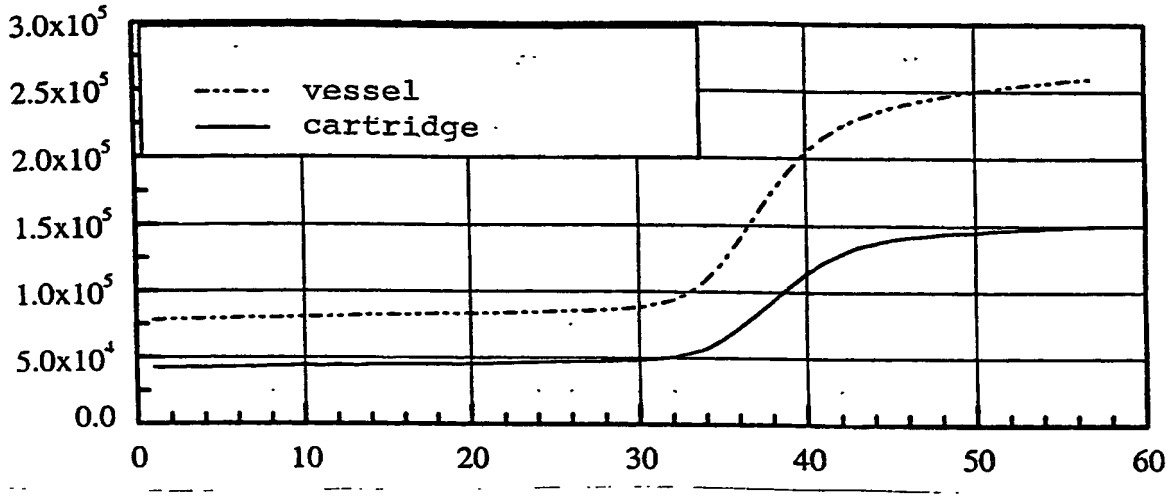


Fig 5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2.0x10^5
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cartridge
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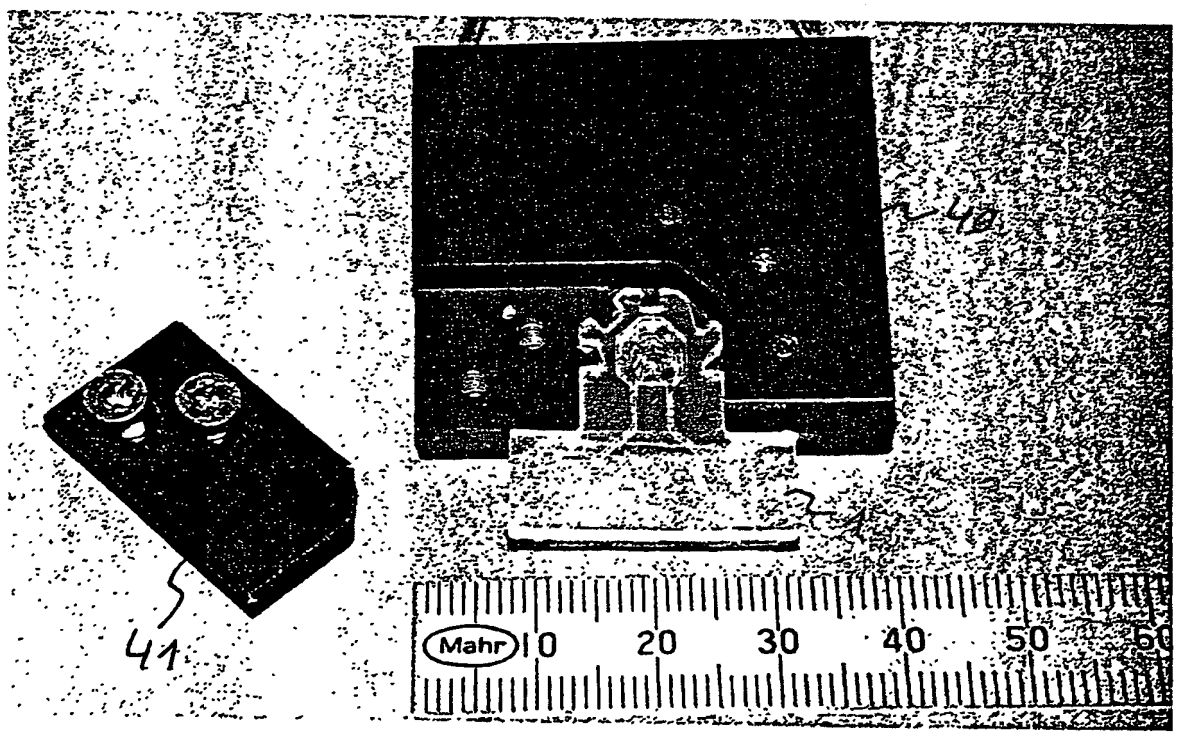
10
20
30
40
50
60

Fig 6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Abstract

System for thermocycling of fluids in cartridges comprising a thermocycling unit in thermal contact with a first substantially planar and heat conducting wall of a cartridge, a light source for transmitting light into the interior of said cartridge through a second light transparent wall of said cartridge which is arranged substantially perpendicular to said first wall, a light detector for detecting light emerging from the interior of the cartridge through said second wall and a fluid providing unit couped to an inlet of the cartridge for providing the cartridge with fluid. The invention further concerns a cartridge for conducting the thermal cycling of fluids.